AN INVESTIGATION OF THE (4;11)(q21;p15)
TRANSLOCATION IN ACUTE LYMPHOCYTIC
LEUKAEMIA

by

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Table of Contents

Table of Contents ................................................................. iv
List of Tables ................................................................. ix
List of Figures ................................................................. ix
Abstract ................................................................. x
Declaration ................................................................. xii
Acknowledgments ............................................................... xiii
Publications ................................................................. xv
Selected conference presentations arising from this thesis ................................................................. xvi
Abbreviations ................................................................. xviii

Chapter 1

Introduction ........................................................................... 2

1.1 Haematopoiesis ......................................................... 2

1.1.1 Classification of the leukaemias .................................................. 3

1.1.1.1 Morphological and cytochemical criteria for leukaemia classification ................................................. 4

1.1.1.2 Immunophenotyping ................................................................. 5

1.1.1.3 Cytogenetics ................................................................. 6

1.1.1.4 Proposed revised classification of the haematological malignancies ......................................................... 6

1.1.2 Incidence of leukaemic subtypes .................................................. 7

1.2 Chromosome translocations and leukaemia .................................................. 9

1.2.1 Chromosome translocations alter normal patterns of gene expression ......................................................... 9

1.2.1.1 Deregulating the expression of a proto-oncogene ................................................................. 10

1.2.1.2 Production of an in-frame fusion gene ................................................................. 11

1.2.2 Common targets of chromosome translocations .................................................. 13

1.2.2.1 Disruption of transcription factors ................................................................. 13

1.2.2.2 Disruption of protein kinases ................................................................. 14

1.2.3 Rearrangements associated with T-cell ALL .................................................. 15

1.3 Identifying genes at chromosome translocation breakpoints .................................................. 17

1.3.1 Positional cloning approach .................................................. 17

1.3.2 Candidate gene approach .................................................. 18

1.4 Aims ........................................................................... 19

1.4.1 Identification of a novel T-cell ALL translocation, t(4;11)(q21;p14-15) .................................................. 20

1.4.2 Preliminary molecular analysis of t(4;11)(q21;p14-15) ........................................................................ 22

1.4.3 Narrowing the chromosome 11 breakpoint region ........................................................................ 23
Chapter 2
Materials and Methods

2.1 Materials................................................................. 25
  2.1.1 Buffers and Solutions:............................................. 25
  2.1.2 Media ...................................................................... 27
2.2 Methods................................................................. 27
  2.2.1 Basic nucleic acid isolation and manipulation procedures ........................................................................ 27
    2.2.1.1 Isolation of Lymphocytes/mononuclear cells from peripheral blood............................................. 27
    2.2.1.2 Thawing of frozen samples ........................................ 28
    2.2.1.3 DNA isolation from cell suspensions ........................................ 28
    2.2.1.4 RNA isolation procedure........................................ 29
    2.2.1.5 Plasmid DNA isolation........................................ 29
    2.2.1.6 DNA ligation...................................................... 30
    2.2.1.7 Preparation of competent E. coli and transformation with plasmid DNA ......................................... 30
    2.2.1.8 Sequencing...................................................... 31
  2.2.2 Cell culture............................................................ 31
    2.2.2.1 Thawing of cells for cell culture .................................... 31
    2.2.2.2 Subculturing of NIH-3T3 mouse fibroblast cells ................................................................. 32
    2.2.2.3 Transfection of NIH3T3 cells with plasmid DNA ................................................................. 32
  2.2.3 Screening the λgt11 bacteriophage library................................. 33
  2.2.4 Southern Analysis.................................................. 34
  2.2.5 Northern Analysis.................................................. 36
  2.2.6 Polymerase chain reaction (PCR) protocols............................... 36
    2.2.6.1 Standard PCR conditions......................................... 36
    2.2.6.2 PCR conditions using Expand™ Long template and Expand™ Hi-Fi PCR System.............................. 37
    2.2.6.3 Sequence of oligonucleotides for hybridisation, PCR and sequencing applications ..................... 37
    2.2.6.4 Restriction endonuclease digestion of PCR products................................................................. 40
    2.2.6.5 PCR product purification........................................ 40
    2.2.6.6 Reverse transcription (RT)-PCR conditions using MMLV ............................................................ 41
    2.2.6.7 Reverse transcription (RT)-PCR conditions using Superscript II ............................................................ 41
    2.2.6.8 DNase I treatment of RNA........................................ 42
  2.2.7 Procedure for 3’ RACE.................................................. 42
Chapter 3
Investigation of the candidate gene, ZNF195

3.1 Confirmation of the 11p15.5 breakpoint region ......................................................... 47
3.2 Subcloning of zinc finger containing sequences from cosmid Z104 ................................. 48
3.3 Are the zinc finger coding sequences present within Z104 transcribed? ......................... 51
  3.3.1 Northern analysis of adult and foetal tissues using the 336 bp Eco RI fragment as a probe 53
  3.3.2 Assessing the specificity of the 336 bp Eco RI fragment for Z104 .............................. 53
  3.3.3 Elevated expression of a 4.3 kb transcript in the t(4;11) patient ................................. 57
3.4 Isolation of lambda phage clones from a HUT-78 cDNA library ................................... 57
3.5 Assembly of restriction fragment and phage sequence into a contiguous sequence ............ 58
3.6 Description of the ZNF195 gene present within Z104 .................................................. 59
  3.6.1 Determination of the intron/exon boundaries of ZNF195 ......................................... 61
  3.6.2 Positioning of ZNF195 within the Z104 cosmid ...................................................... 63
3.7 Alternative splicing of exons 4a and 4b ....................................................................... 64
3.8 Revised Northern analysis with a probe from the 3'UTR of ZNF195 ................................. 66
3.9 Discovery of a deletion/insertion polymorphism in the ZNF195 3'UTR .............................. 69
3.10 Assessing the imprinting status of ZNF195 .................................................................. 71
3.11 Discussion .................................................................................................................... 75
  3.11.1 Northern analysis of ZNF195 expression ................................................................. 75
  3.11.2 Alternative splicing of ZNF195 .............................................................................. 76
  3.11.3 What are the functions of ZNF195? ....................................................................... 76
  3.11.4 Genomic position of ZNF195 .................................................................................. 77
  3.11.5 Is ZNF195 the 11p15.5 breakpoint gene in t(4;11)? .................................................. 78

Chapter 4
Identification of the t(4;11)(q21;p15) breakpoint genes ......................................................... 81

4.1 Investigating NUP98 as a candidate breakpoint gene in t(4;11)(q21;p15) ......................... 82
  4.1.1 Northern analysis ....................................................................................................... 82
  4.1.2 Southern analysis ..................................................................................................... 84
  4.1.3 Testing der(4) and der(11) for retention of NUP98 sequences flanking the t(7;11)(p15;p15) breakpoint .............................................................................................................. 85
    4.1.3.1 Primer pairs 5' of the published NUP98 breakpoint ............................................. 86
    4.1.3.2 Primer pairs 3' of the published NUP98 breakpoint ............................................. 87
4.2 Identification of the chromosome 4 breakpoint gene ..................................................... 89
  4.2.1 3' RACE to determine the sequence fused to NUP98 ............................................. 91
    4.2.1.1 Characterisation of smaller RACE products ....................................................... 96

vi
Chapter 5

Cellular localisation of nrg and its components ................................................................. 114

5.1 Creating constructs for studying the cellular location of nrg ........................................... 115

5.1.1 Discovery of a novel exon in the predominant isoform of RAPIGDS1 .................. 117
in PBMC .......................................................................................................................... 117

5.1.1.1 Other sequence variations in RAPIGDS1 .................................................... 121

5.1.2 Three fragment ligation for the creation of the gfp-nrg expression construct ........ 122

5.1.3 NUP98 cloning ....................................................................................................... 124

5.2 Cellular localisation of gfp tagged nup98, nup98t, smgGDS and nrg ....................... 130

5.3 Discussion .................................................................................................................. 133

5.3.1 The predominant isoform of smgGDS in PBMC contains 12 armadillo repeats ...... 133

5.3.2 Localisation of gfp-nup98 ..................................................................................... 134

5.3.3 Localisation of gfp-nup98t ................................................................................... 136

5.3.3.1 Possible disruption of CRM1 mediated nucleocytoplasmic transport ............ 137

5.3.3.2 Possible disruption of RAL1 mediated nucleocytoplasmic transport ............... 139

5.3.4 Localisation of gfp-smgGDS ................................................................................ 140

5.3.5 Localisation of gfp-nrg ......................................................................................... 141

5.3.5.1 Nrg in the nucleus ......................................................................................... 142

5.3.5.2 The role of SMAP ......................................................................................... 146

5.3.5.3 Nrg in the cytoplasm ..................................................................................... 147

5.4 Conclusion .................................................................................................................. 148
Chapter 6

Conclusions and future studies ................................................................. 150

6.1 A common theme for nup98 fusions ....................................................... 151
  6.1.1 Cbp and p300 are key regulators of haematopoietic differentiation .......... 152
    6.1.1.1 Cbp/p300 interacts with amll ................................................. 152
    6.1.1.2 Haploinsufficiency of cbp causes haematopoietic defects ................. 153

6.2 What is the role of the fusion partners of nup98? ................................. 153
  6.2.1 Why is nrg specific to T-cell ALL? ................................................ 155

6.3 Future studies .................................................................................... 156
  6.3.1 Identifying proteins that interact with nrg and pathways affected by nrg 156
    6.3.1.1 A common pathway for T-cell ALL ........................................... 157
  6.3.2 Does nrg effectively cause increased expression/activity of smgGDS? .... 158
  6.3.3 Further studies on nrg localisation .................................................. 159
  6.3.4 Assessing the transforming properties of nrg .................................... 159
    6.3.4.1 in vitro transformation studies .................................................. 160
    6.3.4.2 in vivo transformation studies .................................................. 161

References ............................................................................................... 164

Appendix  Journal Papers ......................................................................... 191
Abstract

This thesis describes the results of an investigation to determine the molecular basis of an uncharacterised (4;11)(q21;p15) translocation in a patient with T-cell acute lymphocytic leukaemia (ALL). The chromosome 11 breakpoint had been narrowed to the region between the markers D11S860 and D11S470.

A new zinc finger gene near the breakpoint region was cloned and characterised. This gene, subsequently named ZNF195, encodes an N terminal KRAB domain and 14 tandemly repeated Krüppel type zinc finger motifs. ZNF195 was subsequently localised distal to D11S470 and therefore distal to the chromosome 11 breakpoint in the (4;11)(q21;p15) translocation. This excluded disruption of ZNF195 by the translocation.

Subsequently the nucleoporin 98 gene (NUP98) was identified at an acute myeloid leukaemia translocation. NUP98 maps distal to D11S470 and therefore within the breakpoint region. Analysis of somatic cell hybrids segregating the t(4;11) translocation chromosomes showed that the chromosome 11 breakpoint occurred within NUP98. The fusion partner of NUP98 was identified as the RAP1GDS1 gene using 3' RACE. In the NUP98-RAP1GDS1 fusion transcript (abbreviated NRG), the 5' end of the NUP98 gene is joined in frame to the coding region of the RAP1GDS1 gene. This joins the phenylalanine-glycine (FG) repeat rich region of nup98 to smgGDS (the most common name for the protein encoded by RAP1GDS1) which largely consists of tandem armadillo repeats. NRG fusion transcripts were detected in the leukaemic cells of two other adult T-cell ALL patients with a t(4;11)(q21;p15) translocation. This is the first report of a NUP98 translocation in lymphocytic leukaemia and the first time that RAP1GDS1 has been implicated in any human malignancy.
The cellular localisation of nrg was determined and compared to that of its protein components nup98t (t for truncated) and smgGDS. cDNAs were cloned into the pEGFP-C2 mammalian expression vector to create green fluorescent protein (gfp) tagged proteins. The location of the gfp tagged proteins within transfected NIH-3T3 mouse fibroblast cells was visualised using confocal microscopy. Gfp-nup98t was located in a punctate pattern around the nuclear envelope. Gfp-smgGDS was found throughout the cytoplasm and was absent from the nucleus. The hybrid protein gfp-nrg was present throughout the cytoplasm but was also visible within specific subnuclear domains. Therefore the formation of nrg results in nuclear localisation of the normally cytoplasmic smgGDS protein. Nup98t has been shown by others to have strong transcriptional transactivation ability while smgGDS has been shown to be important in blocking apoptosis in thymocytes. It is therefore possible that nrg promotes leukemogenesis through two independent pathways. In the nucleus nrg may act to deregulate transcription pathways critical to normal T-cell development, while in the cytoplasm, nrg may act to increase T-cell survival by promoting an anti-apoptotic phenotype.